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BUDDING IN PEROPHORA.¹

GEORGE LEFEVRE.

ALTHOUGH the phenomenon of budding in the group of Tunicata has received the attention of numerous observers for a long time, during the last twelve or fifteen years investigation has been largely confined to the process in the pelagic forms, Salpa, Doliolum, and Pyrosoma. In the ascidians, however, what we have known of the budding, until recently, has been based almost entirely on the observations of earlier workers. Among the latter Kowalewsky (12 and 13), who studied the bud development in Perophora, Didemnum, and Amaroucium, Della Valle (4), in Didemnum, Distaplia, and Botryllus, and finally Seeliger (29) and Van Beneden and Julin (33), in Clavelina, are especially prominent.

Within the last three or four years, however, our knowledge has been greatly enriched by the researches of Pizon (22) and Hjort (8) on Botryllus, of Salensky (27) on Distaplia, and by those of Caullery (1). Although the observations of these investigators agree in many important points, still, in others, notably the origin of the nervous system, great difference of opinion exists.

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While the Marine Laboratory of the Johns Hopkins University was stationed at Beaufort, N. C., during the summer of 1894, I collected material for the purpose of studying the development of the buds of *Perophora viridis* Verrill, which was found there in great abundance. This material I supplemented the following summer at Woods Holl, Mass., while working in the laboratory of the United States Fish Commission.

My main object in undertaking the investigation was to compare the bud development of *Perophora* with that of *Botryllus*, as described by Hjort (8) and others, and especially to determine, if possible, the origin of the nervous system.

The material, which was easily obtained at both places in large quantities, proved to be most excellent for my purpose, as it contained an unlimited supply of buds in all stages of development.

The preserving fluids used were (1) glacial acetic acid, (2) a mixture of eighty parts concentrated corrosive sublimate solution and twenty parts glacial acetic acid, and (3) Perenyi's fluid. The latter reagent gave perhaps the most satisfactory results, although very good preparations were obtained with the sublimate-acetic mixture, when the objects were left in it not longer than ten minutes.

Mayer's haemalum proved to be most serviceable, while borax carmine gave an excellent stain when used after acetic acid or sublimate-acetic, but was of no value for objects fixed in Perenyi's fluid.

I have made use of Patten's method of orientation (21) with great advantage, and have found it of invaluable assistance in manipulating very small buds, which I have been enabled to cut with accuracy in any plane desired.

In studying the bud development of *Perophora* I have employed serial sections of all stages in the sagittal, frontal, and transverse planes, and also a complete series, throughout the entire development, of buds mounted in various positions as total preparations.

The sections were cut from 3 to 5 μ in thickness on a Thome microtome, and all the drawings were made with the aid of a Zeiss camera lucida.

I take much pleasure in acknowledging my indebtedness to Professor Brooks for the kindly interest with which he has followed my work, and for valuable assistance given me. I also desire to express in this place my great appreciation of the many courtesies extended to me by the late Colonel Marshall MacDonald at the station of the United States Fish Commission at Woods Holl.¹

Perophora viridis Verrill.

This ascidian, which is the only species of *Perophora* known to occur on the Atlantic coast of North America, was first found in Vineyard Sound by Verrill (34), and described by him in 1871. A new species, *P. annectens*, has recently been reported from the coast of California by Ritter (23).

Perophora viridis grows luxuriantly below low-water mark on the wharf piles in Little Harbor, Woods Holl, and Vineyard Haven, Martha's Vineyard. I also found it to be equally abundant during the summer months in the harbor of Beaufort, N. C. The colonies form large, thick clusters of a beautiful greenish-yellow color, and usually occur together with other ascidians and with hydroids, bryozoa, sponges, and barnacles, the delicate stolons creeping over and covering the surfaces of everything within reach.

The Rudiment of the Bud.

Budding in *Perophora* was first studied by Kowalewsky (12), whose careful work on this form laid the foundation for all subsequent investigation of the process of budding in the ascidians.

Metschnikoff (18) had already discovered in *Botryllus* that the bud-rudiment consists of two vesicles, one within the other, the outer being derived from the ectoderm and the inner from the peribranchial wall of the parent. He also observed the splitting up of the inner vesicle to form the median branchial sac and the lateral peribranchial spaces, but neither Metschni-

¹ A preliminary account of some of my results was published in the *Johns Hopkins University Circulars*, No. 119, June, 1895.

koff nor Krohn (15), who confirmed these observations on the Botryllus bud, saw at all clearly the details of the development. This was left for Kowalewsky, who described many of the internal processes occurring in the bud development of *Perophora listeri*. He showed that in *Perophora*, also, the young bud is composed of two vesicles, the outer one being derived from the ectoderm and the inner from the partition or cloison of the stolon. According to Kowalewsky's account, the endodermal or inner vesicle becomes divided completely into three portions, the two lateral fusing dorsally and forming the peribranchial cavity, and the median giving rise to the branchial sac. I shall try to show below that in *Perophora viridis*, at all events, the peribranchial cavity is formed by quite a different process.

The origin of the bud as a double-walled vesicle has been verified by all subsequent investigators, and thoroughly established as a type of development common to all ascidians whose budding has been studied.

The outer wall of the vesicle is directly derived from the ectoderm of the parent animal, and becomes the ectoderm of the bud. According to the majority of investigators, this outer layer takes no active part in the further development, but Salensky (27) and Oka (20), as will be pointed out below, maintain that the ectoderm is concerned in the formation of the nervous system.

As to the derivation of the inner wall of the vesicle, the case is not so simple, for in different ascidians this layer may arise from entirely different parts of the parent, coming in some forms from an endodermal, in others from an ectodermal structure. In *Perophora* and *Clavelina* it is derived from the cloison or septum of the stolon, which in the latter, and presumably in the former, is of endodermal origin; in *Didemnum* and *Distaplia* from the wall of the gut, and in the *Polyclinidae* from the endodermal wall of the post-abdomen.

In all the above-mentioned species, then, the inner vesicle of the bud-rudiment is derived from an endodermal structure. In *Botryllus*, however, this inner vesicle is formed directly as an evagination of the outer wall of the peribranchial sac, whose

origin in the ascidian larva has been a question of much dispute. Kowalewsky (11) first showed that in the larva of simple ascidians the peribranchial sacs are formed as two symmetrical ectodermal invaginations, which later fuse together dorsally and surround the branchial sac. Della Valle (4), however, contradicted these results, and maintained an endodermal origin for the peribranchial sacs in both simple and compound ascidians, and Pizon (22) has recently described the sacs as arising from two diverticula from the branchial sac in the larva of *Botryllus*. Kowalewsky's account is borne out by all the later investigation of the subject, with the exception of that of Pizon. Seeliger (29, *Clavelina*), Willey (36, *Ciona*), Salensky (28, *Diplosoma*, *Didemnum*), and Caullery (1, *Distaplia*, *Leptoclinum*, *Glossophorum*, *Circinalium*) agree in their statements that the peribranchial sacs arise in the manner described by Kowalewsky, and are therefore purely ectodermal. It is fairly safe to assume that Pizon is wrong, and that in *Botryllus*, as in all other ascidians studied, the sacs are ectodermal. If this be the case, as the inner vesicle of the bud-rudiment is directly cut off from the outer peribranchial wall in both embryozooid and blastozooid, it follows that all the organs, including the peribranchial sacs, derived from this inner vesicle throughout the entire series of buds proceeding from the larva are ultimately of ectodermal origin.

After this brief review of the state of our knowledge concerning the origin of the bud-rudiment in the ascidians, I shall now describe the process as I have found it to occur in *Perophora viridis*.

The buds arise in a single row along the course of the delicate stolons, which branch profusely and adhere on one side to the surface over which they creep. The branches come off approximately at right angles to the stems from which they proceed; but aside from this characteristic there is no regularity whatever in the method of branching—they arise at unequal intervals, and as each grows out it gives off in its turn still younger shoots, the whole system becoming much tangled and twisted to form a loose felt-work.

Proceeding towards the growing tips of the stolons, both buds and branches are seen to become successively younger.

In Pl. XXX, Fig. 6, is shown a transverse section of a stolon. The outer wall of the hollow cylindrical tube consists of a flattened epithelium, the ectoderm (*ec.*), covered externally by the transparent cellulose test (*t.*). Running from one side to the other, the septum (*pt.*), is seen in cross-section, completely dividing the tube into two compartments or sinuses (*bl.s.*), in which the free cells of the blood lie scattered about. This stolon partition consists of a double lamella made up of flat, attenuated cells, and may be described as a collapsed cylinder, the walls of which are closely pressed together and attached along the upper and lower borders to the inner surface of the ectodermal tube. It divides the stolon longitudinally into halves, and stretches entirely across the lumen, although in preserved material it is usually more or less shrunken away from the outer wall. The two compartments of the stolon are in open communication with the body cavities of the animals, so that a free circulation of blood occurs from one individual to another.

As Kowalewsky (12) has pointed out, the partition ("Scheidewand") does not reach quite to the extreme distal end of the stolon, but there the two spaces or sinuses open into each other. The blood flows in opposite directions in the two sides of the stolon, up one to the tip, around the free end of the partition, and down the other. When the heart beat is reversed, of course the direction of the blood flow in the stolon is changed.

The buds always arise in the plane of the partition, on the side of the stolon opposite to that which is attached to the underlying surface; the line of attachment is, therefore, parallel to and immediately beneath the lower border of the partition. It will be seen further on that the definitive median plane of the bud coincides with the plane of the partition, and hence the latter structure divides the stolon into a right and left half in reference to the parts of the bud, and not into a dorsal and ventral portion, as described by Kowalewsky (12), whose well-known figure of the *Perophora* stolon is consequently misleading. Looking at the lateral surface of the buds, as shown in that figure,¹ the partition would be seen from the flat side and not on edge.

¹ This figure is reproduced in Korschelt and Heider's *Entwicklungsgeschichte*, p. 1366.

The first indication of the appearance of a bud is a slight bulging out of the ectoderm of the stolon at one point, and if a transverse section be taken at this spot, it will be seen that the two layers of the partition have spread widely apart, giving the appearance of a more or less spherical vesicle in section. This is well shown in Pl. XXX, Fig. 7. The walls of the partition are seen to be no longer composed of flat cells, except on the lower side, that is, the side next the surface of attachment, but have become very much thickened by active cell multiplication. The cells, too, of the ectoderm over the thickened portion of the partition have increased in height and are now nearly cuboidal. The partition, where it is swollen out into a vesicle, becomes drawn away at its lower border from the outer wall of the stolon; I do not think that this is due to shrinkage, for it is invariably found, and, moreover, beyond this region on either side, where the partition is still flat, it is seen to stretch clear across the lumen of the stolon.

The ectoderm continues to bulge out more and more, until it forms a hemispherical protuberance on the surface of the stolon. The cells composing the raised portion of the ectoderm do not remain cuboidal, but from now on, as the swelling increases, become gradually flattened again. The thickened portion of the partition keeps pace with the ectodermal evagination, and grows out into the latter; but the thin walls below now come together, and close off the upper portion as a thick-walled vesicle, without, however, severing the connection. This process is readily understood from Pl. XXX, Fig. 8, in which the walls of the lower part of the partition (*pl.*), have become united again.

In Pl. XXIX, Fig. 1, a bud at about this stage is shown from the exterior.

As the ectoderm continues to push out, it becomes constricted where it passes over into the stolonian ectoderm, the constriction being greater at first before and behind than on the sides. The bud-rudiment, which by this time is almost spherical, is sharply marked off from the stolon, and stands either straight out as a round knob from the surface of the latter or is slightly inclined towards its free tip (Fig. 2).

It now represents the characteristic double vesicle of the young ascidian bud; the outer or ectodermal vesicle is directly derived from the ectoderm of the stolon, and the inner or endodermal vesicle, which has become a closed sac, arises by evagination of the thickened dilated portion of the stolon partition. The connection between the partition and the inner vesicle is retained for a long time, and the cavity of the latter is to be regarded as being in communication with the potential cavity of the partition.

A transverse section at this stage (Pl. XXX, Fig. 8) shows the still somewhat cuboidal cells of the ectoderm of the bud-rudiment, and the endodermal vesicle (*in.v.*), with its much thickened walls lying inside. Active cell multiplication has been going on in the latter, which are of nearly uniform thickness, except below, where they pass off gradually into the thin walls of the partition. The cells of the blood are found scattered about between the two vesicles, and it is to be especially noted that many are lying close against the inner surface of the ectoderm and outer surface of the endoderm at numerous points (*m.c.*).

The bud-rudiment does not long remain spherical, but soon becomes elongated by a growth towards the apex of the stolon. It now assumes an oval shape, and one end lies free over the surface of the stolon, beyond the portion which is directly connected with the latter (Pl. XXIX, Fig. 3). It is the free end which is later to be distinguished as the anterior portion of the bud, while by this process of elongation anteriorly the connection with the stolon comes to be left behind in the posterior region. The side next the stolon wall becomes the ventral surface of the bud, and that opposite it the dorsal.

The protuberances of the stolon ectoderm, which Seeliger (29) describes as occurring at the base of the bud-rudiment in *Clavelina*, are not present in *Perophora*; they are merely simple ectodermal sacs filled with blood cells, and do not contain an evagination of the partition. Seeliger calls them "Nahrkamern," and regards them as reservoirs of food material for the developing buds.

Before going on to describe the origin of the various internal

organs, some of which have by this time begun to make their appearance, I wish to say a word about the partition of the stolon.

As the inner vesicle of the bud is formed entirely from this structure, it is a matter of importance to know from what it is derived in the larva. It has never been observed in *Perophora* how the stolon partition arises, although it is usually supposed to be of endodermal origin from its likeness to the similar structure in *Clavelina*. Van Beneden and Julin (33) have shown that in the larva of *Clavelina* it is formed as a direct continuation of the epicardium, which arises as a diverticulum from the posterior wall of the branchial sac, and is, therefore, entirely endodermal. During the month of August, 1895, I made an attempt to discover the origin of the stolon in *Perophora*. Larvae were put into aquaria, through which water was kept constantly flowing, and, although they settled down and underwent the metamorphosis, at the expiration of nearly four weeks not the trace of a stolon sprouted from them. When larvae and young embryozöoids were afterwards sectioned and studied, nothing like an epicardium, such as occurs in *Clavelina*, was found. I am unable, therefore, to throw any light on the origin of the stolon partition in *Perophora*, but it is fairly safe to say that it does not arise in the same way as it does in *Clavelina*.

The Further Development of the Bud.

At the time when the bud-rudiment begins to elongate, or very shortly after, the rudiments of several new structures are laid down. These are (1) the *pericardium*, (2) the *peribranchial sacs*, (3) the *dorsal tube*, (4) the *gut*, and (5) the *ganglion*. They do not all arise simultaneously, and, although the rudiment of the pericardium is the earliest to appear, it will be necessary to describe the formation of the peribranchial sacs first in order to render intelligible certain relations between these and other structures.

The Peribranchial Cavity.

All investigators agree in deriving the peribranchial cavity from the inner vesicle of the bud-rudiment, but the manner in which it arises is not the same in all species of ascidians.

In *Perophora*, *Didemnum*, and *Amaroucium*, according to Kowalewsky (12 and 13), two parallel longitudinal furrows appear on the outside of the inner vesicle, and by gradually deepening finally divide the latter completely into three portions. The two lateral divisions which are thus cut off grow up over the middle one, and fuse to form the median portion of the peribranchial cavity, which now surrounds the branchial sac dorsally and laterally.

Seeliger (29) has described a different method of formation of the peribranchial cavity in *Clavelina*. According to him, the inner vesicle is not divided into three portions, but into two, one of which, the posterior, gives rise to the branchial sac and the gut, while the other forms the whole peribranchial cavity. These results were contradicted by Van Beneden and Julin (33), who maintained that in *Clavelina* the process is the same as that described by Kowalewsky. As Seeliger's view was not founded on an investigation of an uninterrupted series of stages, and as there were wide gaps in his observations at periods which are especially concerned in the formation of the peribranchial cavity, the supposition that his results are wrong is very probable.

In *Distaplia* Salensky (27) has shown, and his results have been confirmed by Hjort and Frl. Bonnevie (10), that the inner vesicle gives rise to two lateral evaginations, which become completely constricted off as separate vesicles, the peribranchial sacs, and, gradually extending dorsally, fuse together on the median line. These sacs are not formed at the same time, but one is given off from the inner vesicle before the other, so that at a very early stage an asymmetry of the bud is produced.

Della Valle (4) described a similar method of formation of the peribranchial cavity for *Botryllus*, but both Pizon (22) and Hjort (8) have conclusively proved his observations to be erroneous. According to these latter authors, both the lateral

and median portions of the peribranchial cavity in this ascidian arise at the same time as a saddle-shaped bag, which is cut off by two longitudinal furrows from a median vesicle, the later branchial sac. Hjort regards this process as a great curtailing of the embryonic development, such as often takes place in buds. Salensky (27) confirms this conclusion, but goes a step further, saying that "die Entwicklung der Peribranchialhöhlen des Botryllus eine Abkürzung nicht nur bezüglich der embryonalen Entwicklung, sondern auch bezüglich der Entwicklung dieser Organe in den Knospen anderer Ascidien darstellt," *Distaplia*, for example. My observations on the development of this structure in *Perophora viridis*, although agreeing with those of Hjort in so far as they show that the peribranchial sacs do not arise separately as closed vesicles which later unite to form the cloacal cavity, indicate that the process is not so simple as that which occurs in *Botryllus*.

If a transverse section of a bud be examined about the time when the elongation spoken of above is just beginning, it will be found that the wall of the inner or endodermal vesicle is no longer of uniform thickness. Pl. XXX, Fig. 9, is drawn from such a section. The ectoderm covering the bud, although it is not shown in the figure, has again become flattened after its temporary thickening, and is now like that of the stolon. The figure clearly shows that the stolon partition is made up of two lamellae, which are continuous below with each other and pass over above into the walls of the inner vesicle.

The important change to be noted, however, is that the wall of the endodermal vesicle on one side, the left, is getting perceptibly thinner than elsewhere, and that the whole vesicle is no longer symmetrically placed with reference to the stolon partition, but is bulging out slightly to the right. This is the first indication of a marked change which is about to take place in the internal relations of the bud-rudiment.

By a peculiar process, which may be described as a transverse or rotatory growth affecting the inner vesicle, the thicker wall of the right side (Fig. 9, *r.w.in.v.*) is carried or pushed down gradually until it comes to lie eventually on the ventral side, that is, the side next to the stolon.

The stolon partition remains stationary, and the displacement or shifting around of the inner vesicle takes place on this as a fixed support. The process might be illustrated by the drooping of a flower to one side on its stem, although the change of position cannot be a purely passive falling over of the vesicle. In Fig. 9 a small collection of cells (*pc.r.*) is seen adhering to the wall of the vesicle high up on the right side, and these, as we shall see below, form the rudiment of the pericardium. This cell mass remains fixed at the same place on the wall, and during the shifting of the vesicle is borne down towards the ventral side, describing in its descent an arc of about 90° . It therefore furnishes a good register of the progress of the displacement of the vesicle.

As the turning proceeds, the difference in thickness between what was at first nearly the whole right side and the rest of the vesicle becomes more marked; consequently, the cells composing the entire vesicle, except in the thicker region, are seen to be growing more and more flattened.

The displacement is most probably brought about by a rapid growth and flattening of the cells composing the greater portion of the vesicle, whereby the actual right side, which is morphologically the ventral side of the vesicle, is shifted or pushed ventrally through 90° .

This process is analogous, at all events, with the rotation or displacement of the pharynx of the *Amphioxus* larva from right to left, although I am not prepared to claim any phylogenetic relation between the two.

By comparing Fig. 9 with Figs. 10 and 11, Pl. XXX, the process can be readily understood. The shifting, however, involves the anterior end of the vesicle only to a slight extent. In this region a difference in thickness of the walls is not observed, and the rudiment of the dorsal tube, which definitively has a median dorsal portion in the anterior end of the bud, arises as a collection of cells almost at the same time as the pericardial rudiment appears, lying a little to the *left* of the mid-dorsal line on the wall of the vesicle (Fig. 13, *d.t.r.*). If the displacement took place to as great an extent anteriorly as posteriorly, it is evident that this cell mass would appear much

further down on the left side; but that the anterior end is slightly rotated is shown by the fact that the rudiment appears not exactly in the median line, where it is eventually brought through the shifting of the vesicle, but somewhat to the *left* of it.

The formation of the peribranchial cavity is associated with this change of position of the endodermal vesicle. In Figs. 10 and 11 it is seen that the lower portion of the vesicle at the point indicated by the line *a* is being bent in, with the result that the wall in this region makes two angles, one directed inward and the other outward (Fig. 11, *a* and *b*). The apex of the latter marks a point on the wall of the vesicle which will have traveled through 90° when the displacement is completed, as its final position will be in the mid-ventral line.

As the inwardly directed fold (Fig. 11, *f.l.pb.s.*) deepens, it gradually divides off a portion of the inner vesicle on the left side, which is connected with the stolon partition; this is the left peribranchial sac (Pl. XXXI, Fig. 20, *l.pbr.s.*). This fold begins somewhat in front of the middle of the vesicle, and, deepening rapidly in this region, gradually extends posteriorly.

As these changes are going on, the connection with the stolon partition is gradually becoming constricted, and is now only present in the posterior half of the bud, while at the same time the ectodermal stalk is also getting narrower. Ritter (24) in a preliminary note on the budding of *Perophora* says that "when the differentiation of the 'endoderm' into the branchial and two peribranchial sacs takes place, it does so in such a way that the developing blastozoöid is connected with the double-walled partition of the stolon, not by the branchial sac, as has been hitherto supposed, but by the left peribranchial sac." He does not, however, describe how this comes about. From an examination of Pl. XXX, Figs. 9, 10, and 11, it is readily understood. The communication of the body cavity of the bud with the blood spaces of the stolon is never completely closed, as there is always a free circulation of blood from the one to the other; but eventually the left peribranchial sac is entirely severed from the stolon partition. I cannot, however, confirm Ritter's statement (*loc. cit.*, p. 367) that this connection is

lost at an early stage, namely, "at a time when the two peribranchial pouches have merely begun to envelop the branchial sac." I find that it persists for a very much longer time, and is still present, although greatly constricted, at a stage when some of the gill slits have been formed and the peribranchial cavity has been wholly separated from the branchial sac. Pl. XXXI, Fig. 24, shows the connection (*r.st.c.*) at such a stage.

The first indication of the right peribranchial sac is a slight longitudinal folding-in of the wall of the inner vesicle some distance up on the right side, which appears after the shifting of the vesicle has begun. This furrow starts a little in front of the anterior termination of the left peribranchial fold, and as it deepens and extends posteriorly, it is gradually carried down towards the ventral side, in the same way as the pericardial rudiment. It is already present at the stage represented in Pl. XXX, Fig. 11, but has not yet reached back far enough to appear in a section which shows the left fold. In Fig. 12, which is taken from the same series of sections, but a little further forward, it is well marked (*f.r.pb.s.*).

As the shifting continues, the inner vesicle tends more and more to assume a symmetrical position. The two peribranchial furrows, which deepen rapidly and run in obliquely to meet each other, do not come together on the dorsal surface of the vesicle, but some distance below it. The result of this is that when the right and left peribranchial sacs are separated from the inner vesicle a median dorsal portion connecting them is cut off at the same time. This median piece, hence, does not arise, as Kowalewsky (12 and 13) describes, from the fusion of the lateral sacs dorsally, but the three portions are formed by one and the same process. We now find a median vesicle, the later pharynx, surrounded dorsally and laterally by a saddle-shaped bag which consists of the dorsal or cloacal and the lateral divisions of the peribranchial cavity. This is essentially the same process as that which Pizon (22) and Hjort (8) have described for *Botryllus*.

In *Perophora* the folds which separate the peribranchial cavity from the inner vesicle do not involve the entire length of the latter, but leave nearly the whole of the anterior half undivided,

as well as a short region at the posterior end of the vesicle. And, further, the whole peribranchial cavity is not constricted off at the same time, but, as stated above, the furrows begin anteriorly and extend gradually back, so that at any given stage the opening of the median vesicle into the peribranchial cavity is much wider in a posterior section than in one further forward.

When the right and left peribranchial sacs are being formed, as just described, a broad pouch or diverticulum grows out from the anterior margin of each, and by degrees spreads over the undivided portion of the original inner vesicle. These pouches are direct continuations of the lateral cavities, and later completely cover the sides of the anterior region of the peribranchial sac, but *they never fuse dorsally*.

Similar extensions are carried out from the posterior margin of the lateral cavities, and though not prominent at first, still, as the bud gets older and increases in length, they attain a considerable size and surround a part of the digestive tract.

The peribranchial cavity now consists of two deep lateral sacs, surrounding the spacious branchial sac, and connected dorsally by a median space, the cloacal cavity or atrium. The lateral sacs are unsymmetrical, however, until quite a late stage, for the *anterior* pouch of the *right* peribranchial sac grows more rapidly and extends further forward than the similar pouch on the left side, while the *posterior* pouch of the *left* side extends further back than the corresponding one on the right. Eventually the two sacs become symmetrical.

The formation of the peribranchial cavity is easily understood from the series of sections represented in Pl. XXXI, Figs. 16-21; these will be rendered more intelligible by a comparison with Pl. XXIX, Fig. 4, which is drawn from a total preparation of a bud at the same stage of development. The sections are taken respectively at the levels indicated by the parallel lines, *a, b, c, d, e, and f*, of Fig. 4. In Pl. XXXI, Fig. 16, line *a* of Fig. 4, the most anterior one of the series, the branchial sac (*br.s.*) is seen by itself, for the extensions of the peribranchial sacs have not reached far enough forward to appear in the section; the hypophyseal tube (*d.t.*) is shown on the dorsal side

of the branchial sac. Fig. 17, line *b*, only includes the anterior extension of the right sac (*r.a.ex.*), for, as just stated above, the pouch on the opposite side lags behind in its growth. Fig. 18, line *c*, represents a section taken just in front of the anterior face of the cloaca, and shows both peribranchial sacs at the level where they are continued forward into their anterior extensions. In Fig. 19, line *d*, the section passes through the anterior portion of the cloaca (*cl.*), which is seen to connect the lateral sacs; the constriction, which will ultimately completely separate the saddle-shaped bag from the median vesicle, has proceeded in this region to a considerable extent, and has greatly narrowed the opening between the peribranchial and branchial cavities. In a section further back (Fig. 20, line *e*), the folds which are forming the peribranchial sacs are much less deep and wider apart; the connection between the left sac and the stolon partition is present in this region (*st.c.*). Finally, Fig. 21, line *f*, represents a section beyond the peribranchial sacs, the posterior pouches of which at this stage have not yet begun to grow out; the section passes behind the connection with the stolon partition, but through the intestine (*int.*), which is seen on the left side.

The further development of the peribranchial cavity from this stage on merely consists in the completion of the constriction, whereby the saddle-shaped bag is completely cut off from the branchial sac, and in the extension of the anterior and posterior pouches of the peribranchial sacs, which finally surround the whole pharynx laterally. These relations are illustrated by the series of sections (Figs. 22–24), which are taken from the same bud. Fig. 22 is a section through the anterior end of the bud, and shows the lateral extensions of the peribranchial sacs (*r.a.ex.* and *l.a.ex.*) surrounding the pharynx (*br.s.*). The peribranchial cavity is now entirely cut off, and its lateral portions are united in the middle region of the bud by the dorsal connecting piece or cloaca; this condition appears in Fig. 23. Beyond the cloaca the posterior pouches of the lateral sacs, which, like the anterior pouches, are not united dorsally, are seen in Fig. 24 (*r.p.ex.* and *l.p.ex.*); the connection of the left sac with the stolon partition in this figure has already

been referred to. By this time the process of displacement is completed, and the definitive symmetrical arrangement of the pharynx and peribranchial cavity is reached. The connection between the left peribranchial sac and the partition of the stolon is nearly severed; it is found in only two sections of this series, one of which is seen in Fig. 24. A total preparation of a bud at about this stage is shown in Pl. XXIX, Fig. 5, which may be readily compared with these sections.

Epicardium.

This structure was first described by Van Beneden and Julin (33) in the buds and larvae of *Clavelina*, and was shown by these authors to be closely connected with the development of the pericardium. It arises as an evagination of the posterior wall of the branchial sac, and a little further back divides into two blind pouches, which remain separate in the buds, but in the embryo unite to form the "cul de sac epicardique" of Van Beneden and Julin; the latter is continued into the stolon to form the double-walled partition. The development of the epicardium will be again referred to in connection with the pericardium, with which it stands in very close relation in some ascidians.

In *Distaplia*, Salensky (27) has described the epicardial sacs as arising in the buds at an early stage by evagination from the posterior end of the inner vesicle; the two sacs are not formed at the same time, and the left one is always larger than the right.

In the buds of the *Polyclinidae* the epicardium is formed in the same way; two small diverticula, a right and a left one, are given off from the posterior end of the branchial sac, from which they afterwards become detached. They soon, however, unite to form a single tube, which is continued out into the post-abdomen, where it is destined to furnish the inner vesicles of the buds produced by transverse constriction of that region of the body.

The existence of an epicardium in *Botryllus* is denied by Hjort (8), but maintained by Pizon (22). According to the

latter, the inner vesicle at a very early stage gives off two anterior lateral diverticula, one on each side, which later form the peribranchial cavity and also two posterior lateral diverticula. These four pouches are at first separate, but soon the two on each side fuse in the middle region of the bud. When the peribranchial cavity is separated from the inner vesicle, the posterior diverticula are cut off at the same time, and now appear as posterior prolongations of this cavity, with which they always remain in free communication. They are what Pizon calls the "*diverticules périviscéraux*," and in later stages completely envelop the digestive tract. From the fact that these pouches arise as two diverticula from the posterior end of the inner vesicle, Pizon regards them as homologous with the epicardial tubes of other ascidians, and states (*loc. cit.*, p. 29) that "la formation de cette cavité périviscérale n'est pas secondaire et qu'elle s'est annoncée, dès le début, par deux petits diverticules postérieurs de la vésicule primitive, en même temps que les diverticules antérieurs correspondants qui engendreront la cavité péribranchiale."

These perivisceral diverticula, however, differ from the epicardial tubes of *Clavelina*, *Distaplia*, and the *Polyclinidae* in that they communicate with the peribranchial cavity.

Hjort (*loc. cit.*, p. 594) states that the "einheitliche Peribranchialblase sich nun derart weiter entwickelt, dass sie nicht nur den Abschnitt des Kiemendarmes, sondern den ganzen Darmtractus unwächst," and Salensky (27), who accepts the conclusion of Pizon as to the homology of the perivisceral diverticula, thinks that Hjort evidently saw the "epicardial sacs" in *Botryllus* but failed to recognize them as such. Salensky believes that the connection of the "epicardial sacs" with the cloaca in *Botryllus* must be regarded as a result of the early separation of the peribranchial cavity from the inner vesicle.

In the light of these considerations it is possible that the posterior extensions of the peribranchial sacs, which I have described as arising in the buds of *Perophora viridis*, are likewise homologous with the epicardial sacs of other ascidians. It is to be remembered, however, that if such be the case, which

I think doubtful, their direct origin from the inner vesicle has been completely lost, as they do not appear until quite a late stage, and then merely as prolongations backward of the lateral portions of the peribranchial cavity, after the latter have been entirely cut off from the inner vesicle. This would, therefore, be a still more modified condition than that which is found in *Botryllus*.

Pizon (*loc. cit.*, p. 105) makes the statement, which is not, however, illustrated by figures, that he has confirmed on the buds of *Perophora listeri* the results of Kowalewsky (13, *Amaroucium proliferum*) and of Van Beneden and Julin (33, *Clavelina rissoana*) in regard to the origin of the epicardial tube. "Ce tube," he says, "résulte bien de la réunion de deux petits diverticules qui naissent à droite et à gauche du sac branchial et qui s'isolent complètement de celui-ci à un moment donné." Such a description is not in the slightest accord with my observations, and if an epicardial tube arises in this manner in the buds of the European *Perophora*, it certainly does not in *Perophora viridis*.

The Branchial Sac or Pharynx.

That portion of the original inner vesicle which is left after the separation of the peribranchial cavity becomes the pharynx. At its anterior end it finally opens to the exterior through the branchial orifice, and after the appearance of the gill slits communicates with the peribranchial cavity, while posteriorly it leads off into the digestive tract.

The formation of the branchial sac in the buds of *Perophora viridis* is complicated by reason of the peculiar shifting of the inner vesicle, which has been described above. The whole vesicle, with the exception of the anterior end, which, as already stated, is but slightly involved in the process, becomes shifted or revolved through about 90°, in such a way that the original right wall of the vesicle comes to lie ultimately on the ventral side. This right wall, as has been shown, is early found to be much thicker than the rest of the vesicle, the difference being due, not to an increase in thickness of this region, but to the

flattening of the cells composing the remaining portion of the vesicle. It is this thickened wall, originally on the right side, which forms the floor of the pharynx in that part of the vesicle which is concerned in the displacement. Very soon after the beginning of the change in position, a shallow longitudinal groove is found on the inner surface of the vesicle in the middle region of the bud, lying on the right side on a level with the lower border of the pericardial rudiment. This is the first appearance of the *endostyle*; its position is shown at *end* in Pl. XXX, Fig. 11, but at an earlier stage it is found much higher up. In this figure and the next one it is seen that the groove runs through about the middle of the thickened area, that is to say, above and below it there are equal portions of the thick wall, which will lie to its right and left when the change in position of the vesicle is fully accomplished.

The groove rapidly extends anteriorly and posteriorly, and at the same time becomes deeper and broader. When it reaches its definitive position in the mid-ventral line, it stretches throughout the entire length of the branchial sac.

It will not be necessary to speak of the differentiation of the endostyle into the various zones of cells which go to make it up, as these have been described by numerous authors, — Della Valle (3), Herdman (7), Lahille (17), and others.

It is to be especially noticed in Figs. 10, 11, 20, and 24 that the positions of the pericardial rudiment and endostylar groove in reference to each other remain the same during the displacement of the vesicle. From this fact it is evident that the thick portion of the vesicle is carried down bodily, and that no interstitial growth takes place in this region during the process, else the distance between the pericardium and endostyle would not remain the same. It cannot be said that the pericardial rudiment might compensate by its own growth for any increase in extent of that part of the wall against which it lies, for it covers practically about the same area as long as it adheres to the vesicle. It would seem, therefore, that the change in position of the inner vesicle is brought about by the stretching out and flattening of the cells in all but the thick area, and that the latter is borne or rather pushed down toward the ventral side.

The Branchial Stigmata.

The branchial stigmata or gill slits are not formed until after the peribranchial cavity has been completely separated from the branchial sac. The first to appear lie far back towards the posterior end, but very soon they begin to break out in spots all over the sides of the branchial sac.

The tendency to arise in vertical rows becomes apparent when only very few are present, but each slit is a separate and independent formation. I have never observed the origin of one slit from another, such as occurs in the larvae of ascidians.

Fig. 5, Pl. XXIX, represents a stage when about eight slits have been formed on each side; as the anterior pouches of the peribranchial sacs grow further and further forward, new rows of slits are laid down along their free margins.

The first indication of a gill slit is a small, circular, thickened area of the branchial wall, which at this spot becomes slightly evaginated until it touches the visceral wall of the peribranchial sac (Pl. XXXII, Fig. 29 *a*, *g.s.r.*). The cells of the latter at this point become thickened somewhat, and now a fusion takes place between the two walls; this is seen in Fig. 29 *b* (*g.s.r.*). The opening, which breaks through the center of the fused patch of cells, is drawn out later in the long axis of the bud into a narrow slit, which is provided with cilia in the usual way. The upper part of Fig. 29 *b* shows a slit just after the opening has been formed (*g.s.*).

The Branchial and Cloacal Orifices.

The branchial orifice arises at a tolerably late stage, and is first indicated by a great increase in thickness of the ectoderm at a point opposite the extreme anterior end of the branchial sac. This thickened area becomes invaginated until the bottom of the pit touches the endodermal wall, and a complete fusion of the two soon takes place (Pl. XXXII, Fig. 28 *a* and *b*, and Pl. XXIX, Fig. 5, *e.br.o.*). The cells in the center of the fused area break down, and the cavity of the pharynx is put into communication with the outside. As is shown in Fig. 28

a and *b*, many mesodermal cells (*ml.c.*) attach themselves to the inner surface of the ectodermal depression, become greatly elongated, and are eventually transformed into muscle fibers.

In *Botryllus*, according to Pizon (22), it is the branchial wall which thickens and evaginates to fuse with the ectoderm, while the latter plays but a small part in the production of the orifice. The process, as it occurs in *Perophora viridis*, is quite similar to that described by Kowalewsky (11) for *Phallusia*, and by Van Beneden and Julin (33) for *Clavelina*.

The cloacal orifice is formed in exactly the same manner, by the union of an ectodermal invagination with the dorsal wall of the cloaca (Pl. XXIX, Fig. 5, and Pl. XXXI, Fig. 23, *e.cl.o.*).

It will not be necessary to speak here of the various appendages and ciliated growths of the pharynx which arise later, — namely, the tentacles, papillae, languets, dorsal lamina, and peripharyngeal bands, — as these are merely differentiations of the pharyngeal epithelium, and have been sufficiently described by numerous authors.

The Digestive Tract.

Some time before the displacement of the inner vesicle is completed, and when the folds which will cut off the peribranchial cavity are not very deep, the wall of the inner vesicle high up on the left side at the extreme posterior end becomes much thickened, and soon evaginates to produce a little blind pouch, the rudiment of the digestive tract (Pl. XXX, Fig. 15, *gt.r.*). This lateral diverticulum grows out as a tube, which at once bends sharply downwards and forwards, while, as the shifting of the vesicle continues, its opening into the latter is carried up nearer and nearer the mid-dorsal line, where it will ultimately come to lie. The tube soon turns abruptly on itself to form a close U, and, now growing upward along the outer wall of the left peribranchial sac until it reaches the dorsal surface, finally bends directly forward, and stops short at the posterior wall of the cloacal cavity. At this point the distal extremity of the tube fuses with the cloacal wall, an opening breaks through, and the anus is established. The differentiation into

oesophagus, stomach, and intestine takes place very early, and is apparent at a stage considerably younger than that shown in Pl. XXIX, Fig. 4. The course and development of the tube are sufficiently illustrated by Figs. 4 and 5.

As the bud grows and increases in length, the digestive tract enlarges enormously, the U becomes opened more and more, and the intestine describes a wide curve which lies well forward against the outer wall of the left peribranchial sac (Fig. 5). With the anterior extension of the digestive tract and the posterior prolongation of the left peribranchial sac, the whole tract, which lies entirely on the left side of the bud, comes eventually to be closely enveloped by the outer wall of the peribranchial cavity.

The "pyloric gland" or "organe réfringent" of Giard arises as a tubular diverticulum from the lower anterior face of the enlarged portion of the digestive tract which will become the stomach. Before reaching the intestine the tube bifurcates, and each branch in its turn gives off two others, which also divide, the whole system of dichotomously branching tubules finally forming a lace work surrounding the whole intestine.

The development of this problematical organ is already well advanced at the stage shown in Pl. XXIX, Fig. 5 (*o.r.r.*). This figure, together with Pl. XXXII, Fig. 30, which shows a portion of the stomach wall (*st.w.*), leaves no doubt that the tube is directly derived from the digestive tract. This origin was maintained by Della Valle (3), but denied by Roule (25), who stated that the "organe réfringent" is not a part of the digestive tract, but communicates with the heart, and therefore belongs to the vascular system, an opinion already held by Kuppfer (16).

Della Valle's view is also supported by Pizon (22), whose description of the development of the organ in Botryllus agrees minutely with my observations on Perophora.

The terminal branches of the system of tubules which ramify over the surface of the intestine end in little enlargements or ampullae, the walls of which are very thin, and lie closely pressed against the intestinal wall. The cells of the duct are cylindrical, and gradually pass over into the flat cells

of the ampullae. I have failed to find any cilia on the latter, as Chandelon (2) has described in *Perophora*. Pl. XXXII, Fig. 31, shows a cross-section of the intestine (*int.*), surrounded by the thin-walled tubules and ampullae (*amp.*), the flat cells of which contain very deeply stained nuclei. On one side of the figure one of the ducts is cut longitudinally, just where it forks near the surface of the intestine (*o.r.d.*).

Different views have been held concerning the function of the "organe réfringent." Krohn (15), Kupffer (16), and Giard (6) have regarded it as a renal organ; but as the ampullae always contain a clear, unstainable fluid, and never concretions or epithelial débris, this view has been discarded. A second hypothesis, that it is a digestive gland which gives its secretion to the intestine, has been held by Chandelon (2) and Della Valle (3), the latter attributing to the organ an hepato-pancreatic function. Pizon (22), however, believes that the flat cells of the ampullae possess no glandular characters, and cannot be reconciled with a secretory function; but he is inclined to regard the organ as a *chyliferous apparatus*. He says (*loc. cit.*, p. 96): "Je suis plutôt porté à croire que l'épithélium des ampoules ne sécrète rien, et qu'il se charge simplement d'absorber les produits de la digestion qui sont assimilables et qui n'ont pas été pris par les parois de l'intestin. Ces produits quitteraient ensuite la cellule pour aller se mélanger au sang, dont les corpuscules sont précisément extrêmement nombreux autour des ampoules terminales." Although Pizon's hypothesis would seem the most probable one, as the histological structure of the organ is not such as to suggest a glandular function, still, the rôle played by the "organe réfringent" must remain uncertain until the nature of the liquid contained in the tubules is determined.

The Pericardium and Heart.

Concerning the origin of the common rudiment of the pericardium and heart, investigators have given widely divergent accounts, some deriving it from endoderm, others from mesoderm. Although it is very certain that this structure arises

differently in different ascidians, still, in the buds of one and the same form statements of authors are at variance.

Seeliger (29) describes the pericardium as arising in the buds of *Clavelina* from an enormously large evagination of the ventral portion of the branchial sac, which later becomes separated as an independent vesicle. He did not, however, distinguish the epicardial sacs, and mistook a part of the latter for the pericardium. Van Beneden and Julin (33) showed conclusively that the diverticulum of the branchial sac, observed by Seeliger and called by him the pericardium, is merely a part of the stolon partition wall and is not concerned in the formation of the heart. According to the Belgian authors, who described in detail the development of the pericardium in the buds of *Clavelina*, the pericardium and epicardium at first form a common cavity with the inner vesicle. A separation takes place later in such a way that the epicardium remains in communication with the inner vesicle, while the pericardium becomes entirely cut off from the latter, but retains its connection with the stolon partition. Van Beneden and Julin maintain that the union of the epicardium with the branchial sac is never lost in the bud development of *Clavelina*, and, therefore, do not agree with Seeliger's statement that the diverticulum, which he observed and erroneously regarded as the pericardium, becomes separated from the branchial sac. Seeliger's description of the early constriction of the inner vesicle from the stolon partition is not confirmed by the Belgians, who showed that the pericardium, originally a part of the inner vesicle, preserves its connection with the partition wall, as explained above.

A somewhat similar origin of the pericardium, together with the epicardium from the inner vesicle, is stated by Pizon (22) to occur in the *Polyclinidae*; for example, in *Circinalium* and *Amaroucium*.

Our knowledge of the derivation of this structure in the buds of *Botryllus* is very much less certain. Pizon (22) declares that the pericardium arises as a little diverticulum from the lower wall of the inner vesicle, which becomes completely constricted off as an elongated tube. His conclusion as to the endodermal

origin of the peribranchial rudiment cannot, however, be unhesitatingly accepted, since his figures do not satisfactorily establish the correctness of his description, while the supposition that he has not followed the development with sufficient care is very strong. Salensky (27, p. 527) calls attention to the fact that the little circle of epithelial cells which Pizon marks with the letters *Per* in Pl. I, Fig. 7, "wohl auch einen Querschnitt der unteren Wand des Kleimendarmes darstellen kann," and that it is not at all proved that it is the same structure as the pericardium, figured in later stages.

The first appearance of the pericardial rudiment observed by Hjort (8) in *Botryllus* was a small clump of cells lying against the ventral wall of the inner vesicle in the posterior part of the bud to the right of the middle line. As to the derivation of these cells, Hjort was unable to say whether they were mesodermal cells or cells which had wandered out from the endoderm, but he distinctly states that an evagination of the inner vesicle does not occur at this point.

In the buds of *Distaplia*, Salensky (27) observed a similar collection of cells lying against the lower wall of the branchial sac and surrounded by mesodermal cells. He maintains that there is no ground for attributing an endodermal origin to the rudiment, which is from the beginning sharply marked off from the wall of the branchial sac, and he therefore concludes that the pericardium is derived from the mesoderm.

The result to which my observations on the bud development of *Perophora viridis* have led me, in regard to the origin of the pericardial rudiment, is in accord with that of Salensky.

At about the stage represented in Pl. XXX, Fig. 9, a very loose patch of cells (*pc.r.*) is found applied to the outer surface of the inner vesicle high up on the right side in the posterior end of the bud. Before this time many isolated cells are seen adhering to the wall of the vesicle at numerous points (Figs. 7, 8), but when the difference in thickness between the right side and the rest of the vesicle is just becoming apparent a marked tendency in the scattered cells to accumulate in one spot is noticed. At first there is but a single layer of cells joined loosely together end to end and forming a somewhat elongated

patch; this is the rudiment of the pericardium, which is the first organ to make its appearance. In Pl. XXXII, Fig. 25 *a*, which is drawn from a frontal section, an extremely early stage is shown at *pc.r.* That the rudiment is formed by the coming together of *free amoeboid cells of the blood* I believe there is no reason for doubting. At the stage represented in this figure, the similarity between many of the cells scattered freely about in the space between the ectoderm and endoderm and those which form this cell mass is perfectly apparent. There is certainly not the slightest evidence that the wall of the inner vesicle evaginates or its cells proliferate at this point; the line of demarcation between the two structures is distinct throughout, and shows no interruption in its continuity.

The rudiment does not long remain of one layer, but by the addition of other cells and by active cell division it soon becomes thicker and more compact (Fig. 25 *b*). The cell boundaries are gradually lost, and the solid mass is now firmly attached to the wall of the vesicle (Fig. 25 *c*). The rudiment, which has now an elongated form, is not in a horizontal position, but posteriorly is at a higher level than anteriorly.

When the shifting of the inner vesicle begins, the clump of cells is borne passively down towards the ventral side, but long before it has reached its definitive position a cavity has appeared in its center, around which the cells become arranged in an epithelium to form an elongated closed sac (Figs. 25 *d* and *e*).

The position of the rudiment at various stages during its descent has already been observed while considering the displacement of the inner vesicle from Pl. XXX, Figs. 9, 10, 11, and Pl. XXXI, Figs. 20, 24.

About the time that this change in position is accomplished the pericardial sac loses its attachment to the branchial wall, and grows considerably longer and wider. The cells composing the sac become very much flattened and attenuated, except in the dorsal wall, which is soon folded in longitudinally to form the heart in the usual way (Fig. 24, *d.w.pc.s.*). The pericardium in its definitive position is placed under the posterior floor of the pharynx, just to the right of the median line. It is not horizontal, *i.e.*, parallel with the surface of the stolon,

but the posterior is higher than the anterior end. This inclination is seen in Pl. XXIX, Figs. 4, 5, in which the pericardium is indicated at *pc*. In the latter figure, which shows about its final position, the pericardium is seen to extend from a point at a level nearly as high as the upper end of the stomach straight down to the stalk which connects the bud with the stolon.

The Dorsal Tube and Ganglion.

Of all the organs of the ascidian bud, that which has given rise to the greatest amount of discussion is the nervous system. Its origin and development have been matters of much dispute, and so wide is the difference of opinion concerning points of fundamental importance that there is little hope at present of harmonizing the conflicting statements of various authors.

A close relation between the dorsal tube and ganglion has been affirmed by many who hold to a common origin of the two, but is strenuously denied by others, who assert that the dorsal tube arises independently and has nothing whatever to do with the nervous system. Different authors have ascribed to these structures an ectodermal, a mesodermal, and an endodermal origin, and have thereby exhausted the entire series of possibilities.

Kowalewsky (12), for the buds of *Perophora*, was the first to describe an endodermal origin of the nervous system. According to him, the dorsal wall of the branchial sac evaginates to form a tube, which retains its connection with the branchial cavity, and which he calls the "Nervenrohr." In his later work on the budding of ascidians (13) he describes the rudiment of the nervous system in *Amaroucium* and *Didemnum* as "ein sehr langes, am vordern Ende ziemlich breites Rohr, dessen Lumen mit der Höhle des Kiemensackes zu communiciren scheint" (*l.c.*, p. 465). He did not follow the development of this tube, which he held to be derived from the endodermal vesicle, and was ignorant of its relation to the nervous system of the adult animal. It is probable, however, that he saw the ganglion in *Amaroucium*, at least, but failed to recognize it, for he says (*l.c.*, p. 465), "Bemerkenswerth ist noch, dass über dem Nerven-

rohr sich eine Anhäufung von sehr blassen Zellen befindet, welche bei weiterer Entwicklung zu verschwinden scheinen." *Amaroucium* is one of those ascidians in which the ganglion lies *above* the hypophyseal tube.

Ganin (5), who studied the bud development in *Didemnum* and *Botryllus*, derived the nervous system from a vesicle which he described as being cut off from the inner vesicle of the bud and converted into a long cylindrical tube lying over the dorsal wall of the branchial sac. The ganglion, according to Ganin, becomes differentiated from a part of this tube, the remainder of which forms a ciliated organ communicating with the branchial cavity. His description is very obscure, however, and the only points to be noticed are that the dorsal tube, according to this author, is derived from the endodermal vesicle and that it gives rise to the ganglion.

Giard (6) and Della Valle (3 and 4), who studied the bud development in different species of ascidians, contributed nothing of value concerning the nervous system, but both ascribe a common origin to the dorsal tube and ganglion.

The views of Seeliger (29) are very different from the foregoing. According to him, the dorsal tube and ganglion in the buds of *Clavelina* arise from a common rudiment, which is derived from mesodermal cells. This belief was not based on direct observation, since he did not examine sufficiently young stages, but was arrived at through theoretical considerations. The great similarity between the individual cells of the nerve-rudiment and the free blood cells in the body cavity of the bud Seeliger holds is good evidence for the mesodermal origin of this structure. He furthermore points out that the cells composing the ganglion of the larva would be carried off in the blood after the disintegration of that organ, and give rise in the bud to some of these free cells. The latter would, therefore, be "*directe Abkömmlinge eines frühere gangliösen Organs*," and it would be but natural for them to resume the function which they had once possessed. Van Beneden and Julin (33), on the contrary, in their work on the development of the buds of *Clavelina*, state that the nervous system is derived from the ectoderm, and first appears as a cord of cells lying close against

the ectodermal wall. Their description, however, is very incomplete and unsatisfactory.

Our more recent knowledge of the subject is due to the researches of Pizon (22), Oka (20), and Hjort (8) on *Botryllus*, of Salensky (27) on *Distaplia*, and of Caullery (1) on *Glossophorum* and *Diplosoma*. The first three authors, although they are in agreement concerning the origin of the dorsal tube, differ widely in respect to the derivation of the ganglion. According to all three, the dorsal tube in the *Botryllus* bud arises as an anteriorly directed evagination of the peribranchial cavity, ending blindly in front, but freely opening into the cavity at its posterior extremity. This tube grows forward, and its anterior end fuses with the wall of the branchial sac, whose cavity is then put into communication with the lumen of the tube, while the posterior connection becomes obliterated. The definitive opening of the hypophyseal tube is, therefore, secondary. So far we find these authors agreeing, but it is quite otherwise when we come to consider the origin of the ganglion.

Pizon maintains that the ganglion of the bud is derived directly from a fine nerve string, which grows out from the ganglion of the parent bud, or, in the first place, from that of the larva into the young bud. His view is not based on actual observation, and his arguments, which are far from satisfactory, fail to convince. He avers that a constriction of the ganglion from the wall of the dorsal tube does not take place, but, on the contrary, the figures of Hjort (8, *Botryllus*) prove fairly conclusively that such a constriction does actually occur. Hjort's contention that the ganglion is formed from the thickened ventral wall of the hypophyseal tube is based on a study of an unbroken series of stages and is clearly borne out by his figures. In a short note¹ on the budding of *Botryllus*, which was published recently, I added additional evidence in support of Hjort's view, and reproduced a drawing which showed beyond a doubt that the thickened ventral wall of the dorsal tube is pinched off to form the ganglion.

The account given by Oka is entirely different. According

¹ *Johns Hopkins University Circulars*, No. 119, June, 1895.

to this observer, cells wander out from the ectoderm, fasten themselves to the ventral wall of the dorsal tube, and there form the ganglion. These wandering ectodermal cells were also observed by Pizon, who described them as giving rise to a portion of the genital gland, to muscular fibers, and to certain cells of the blood. The principal difference, then, between the three authors is that, whereas Pizon and Oka hold to an independent origin of hypophysis and ganglion, Hjort maintains that there is a common rudiment for the two structures.

The results of Salensky (27) on the bud development of *Distaplia* do not stand in the slightest agreement with any of those obtained for *Botryllus*. The nervous system of the *Distaplia* bud is of ectodermal origin, according to him. Cells sink down at a very early period from the ectodermal wall and form a solid mass, which later acquires a cavity, increases in length, and produces a tube. The latter becomes differentiated into three parts; the anterior gives rise to the hypophysis, the middle to the ganglion, and the posterior portion to the visceral nerve. The hypophysis and ganglion have, therefore, a common origin. This mode of formation, however, is only true of the primordial bud; for all the other buds, which are produced from it by fission, derive their nervous system by division directly from that of the parent along with the rest of their organs.

It might be mentioned that in the buds of *Pyrosoma*, Salensky (26) has described a similar ectodermal origin of the nervous system, although Seeliger (30) in the same form derives the common rudiment of ganglion and hypophysis from mesodermal cells.

Salensky's results on *Distaplia* are directly contradicted by Hjort and Frl. Bonnevie (10). The latter find no trace of the nervous system in the early stage at which Salensky describes its first appearance, but maintain, on the contrary, that a forwardly directed diverticulum is later formed from the dorsal wall of the inner vesicle, just as in *Botryllus*, and that the ganglion is differentiated from the wall of the dorsal tube.

Pizon (22), in his work on *Botryllus*, states that he has made observations on the development of the dorsal tube in the buds of a number of other ascidians. In *Perophora* and *Clavelina* he

observed the tube over the dorsal wall of the branchial sac, but did not obtain stages which were young enough to enable him to determine its origin. He concludes, however, on the insufficient evidence of Kowalewsky's observations on *Perophora*, that the dorsal tube arises as a diverticulum of the endodermal wall. Since, in *Clavelina*, the later stages in the development of the dorsal tube are similar to those of *Perophora*, he holds that in this ascidian, also, the origin is the same. In two of the Polyclinidae, viz., *Amaroucium proliferum* and *Circinalium*, in *Didemnum niveum* and in *Astellium spongiforme*, he has observed the dorsal tube arising as an endodermal diverticulum, which acquires a secondary opening into the branchial sac at its anterior extremity, just as in *Botryllus*. In none of these forms did he determine the origin of the ganglion, but he comes to the unwarranted conclusion that this structure is derived, independently of the dorsal tube, in the same way as he has described for the Botryllidae.

Hjört (9) has recently studied the development of the neuro-hypophyseal system in the buds of *Glossophorum sabulosum*, one of the Polyclinidae, and Caullery (1) in *Glossophorum luteum*, *Circinalium conrescens*, and *Diplosoma gelatinosum*, and, although both of these authors find that the dorsal tube arises in the manner described by Pizon, that is, as an anteriorly directed endodermal diverticulum, they give a different account of the origin of the ganglion. In all the species studied the ganglion is formed as a differentiation of the dorsal wall of the hypophyseal tube, and has, therefore, a common rudiment with the latter. Their results are in agreement with what Hjort has found in *Botryllus*, except that in all of the above-mentioned ascidians the hypophysis lies below the ganglion, whereas in *Botryllus* it is above.

Finally, Ritter (24), who has recently described the bud development of *Goodsiria*, a genus in which budding had not been observed before, finds a complete agreement, concerning the origin of the neuro-hypophyseal system, with Hjort's work on *Botryllus*.

In the same paper Ritter gives a preliminary account of some observations on the development of the buds of *Perophora*

annectens and *P. listeri*. In both species he derives the common rudiment of the hypophysis and ganglion from cells which wander out from the dorsal wall of the inner vesicle.

After this short review of the state of our knowledge regarding this much confused subject, I shall now give an account of my own observations on the development of the dorsal tube and ganglion in the buds of *Perophora viridis*.

My results, which are based on a study of an uninterrupted series of stages, have led me to believe that the conclusion which Seeliger drew from purely theoretical considerations concerning the origin of these structures in *Clavelina* is also true of *Perophora viridis*. I shall try to show that *the dorsal tube and ganglion are derived from amoeboid cells of the blood*.

The dorsal tube is formed long before the ganglion, and the rudiment from which it will arise is first indicated just after the collection of cells which is to produce the pericardium makes its appearance. When the difference in thickness between the right side and the rest of the vesicle is becoming apparent — hence at a time when the rotation is about beginning — in the anterior portion of the bud, a little to the left of the median dorsal line, there is seen an irregular elongated patch of cells very loosely grouped together and lying on the outer surface of the inner vesicle (Pl. XXX, Fig. 13, *d.t.r.*). The free amoeboid cells in the space between the ectoderm and endoderm are especially numerous in this region, and are closely associated with the collection of cells adhering to the vesicle. Although I have examined my sections with the greatest care under an oil-immersion lens, from the very first appearance of the rudiment, I have failed to find any indication of cell migration from the endodermal wall, and, therefore, cannot confirm Ritter's statement (*l.c.*, p. 368) that an "indistinguishable transition from the cells of the 'endoderm' to those of the neuro-hypophyseal anlage is to be traced," and also that cells can be found "in the act of migrating from the 'endoderm' into the anlage."

The line of separation between the rudiment and the wall of the vesicle is seen to be perfectly distinct and clearly marked,

and there is no evidence whatever of proliferation of endodermal cells at any point (Pl. XXXII, Fig. 26, *a*). During the early stages of development the cells which are to form the dorsal tube and many of the blood cells are absolutely identical in appearance and exhibit the same amoeboid character. So gradual is the transition from the free blood cells to the cells of the rudiment that it is at first impossible to say where the former end and the latter begin (Fig. 26, *b*).

I believe, therefore, that all the evidence shows that *the dorsal tube is derived from free amoeboid cells of the blood*.

By further additions from outside, and by active cell multiplication within the mass, the rudiment gradually increases in size; its cells become more closely packed together, and soon form an elongated solid cord, lying close against the dorsal wall of the vesicle in the anterior end of the bud (Fig. 26, *c*). Fig. 26, *a*, *b*, and *c*, illustrates the development up to this point; *a* is drawn from the same section as Pl. XXX, Fig. 13; *b* and *c* from the series to which Figs. 10 and 11 respectively belong. It has been stated above that the anterior portion of the inner vesicle is only slightly involved in the displacement already described; but that it is to a certain extent is proved by the fact that the rudiment of the dorsal tube first appears not exactly in the mid-dorsal line, but a little to the left of this (Fig. 13, *d.t.r.*). By the time the shifting of the vesicle has proceeded somewhat further than is shown in Fig. 11, the string of cells, which is now solid, has been carried up to the median plane.

Very shortly after it has reached its definitive position, a lumen appears in the center of the rudiment throughout its entire length, and around this the cells become arranged into a one-layered epithelium (Pl. XXXII, Fig. 26, *d* and *e*).

By following the course of development up to this point, it is seen how an epithelial tube does actually arise from free mesenchymatous cells, — a thing which Hjort has characterized as most improbable. In criticising Seeliger's view of the origin of the neuro-hypophyseal system in *Clavelina*, this author says (8: p. 602): "Die Wahrscheinlichkeit dafür, dass ein Ganglion und ein epitheliales Rohr sich aus zusammengehäuften

Mesodermzellen bilden sollte, scheint mir so gering zu sein, wie für die Auffassung Herdman's, dass die innere Blase der Knospenanlage einen solchen Ursprung habe."

About the time that the peribranchial cavity is completely cut off from the inner vesicle, the anterior extremity of the dorsal tube fuses with the dorsal wall of the branchial sac, an opening breaks through, and the lumen of the tube is put into communication with the branchial cavity. The posterior end of the tube abuts against the anterior wall of the cloaca, but never opens into the latter, in contrast with the condition found in *Botryllus* and many other ascidians.

Pl. XXX, Fig. 14, represents a median sagittal section of a bud before the complete separation of the peribranchial cavity, and, therefore, before the dorsal tube has acquired an opening into the branchial sac. The section passes through the entire length of the tube (*d.t.*), which is seen to be closed at both ends and made up of an epithelium of one layer.

In my preliminary work on the budding of *Perophora*, already referred to, I made the statement that "the ganglion is formed by a thickening of the *dorsal* wall of the tube, which eventually becomes constricted off in the manner described by Hjort for *Botryllus*, although in the latter it is the *ventral* wall of the tube which gives rise to the ganglion." More careful study of very young stages, however, has convinced me that the above is not an accurate description of the formation of the ganglion.

After the communication between the dorsal tube and branchial sac has been established, a few cells, identical in appearance with the amoeboid blood cells, are found adhering to the dorsal surface of the tube throughout the greater part of its length; this elongated, loose patch of cells constitutes the rudiment of the ganglion (Pl. XXXII, Fig. 27, *a*, *g.l.r.*).

It is a difficult question to decide whether these cells are entirely cells of the blood, as their appearance indicates, or whether they are derived by proliferation in the wall of the tube, for in many places the boundary line of the latter is broken, and there is no sharp demarcation between the cells of the rudiment and those of the tube, as seen in Fig. 27, *a*. Many sections, however, such as the one shown in Fig. 27, *b*,

which represents a slightly older stage, leave little room for doubt that nuclei do wander out into the rudiment. But, on the other hand, I think that this figure and Fig. 27, *c*, show equally well that blood cells are added to the mass from the outside.

I have, therefore, come to the conclusion that *the ganglion has a double origin, and that the wall of the tube and free amoeboid cells coöperate in forming it. But, as we have seen that the dorsal tube is made up of cells of the blood, it is, therefore, to be remembered that the ganglion is ultimately derived solely from this source.*

The ganglionic rudiment is at first a very irregular heap of cells, and is closely associated at the periphery with surrounding blood cells. The cell boundaries are completely lost very early, and the mass rapidly increases in size by multiplication of nuclei within, by further acquisition of cells from without, and by continued migration of nuclei from the wall of the tube (Fig. 27, *c*). The nuclei now arrange themselves in a couple of layers around a central core, in which fine fibrils are laid down, and the ganglion becomes completely marked off from the wall of the tube; the definitive structure is now attained. Fig. 27, *d* and *e*, illustrates the later course of development.

My observations, therefore, have led me to believe that the hypophyseal tube and the ganglion are formed only in part from a common rudiment, and in this respect to take a middle ground between Hjort, Salensky, and Caullery, on the one hand, who have described a common origin for these structures in the ascidians studied by them, and Oka and Pizon, on the other, who maintain that they arise independently. In deriving the hypothesis and ganglion, however, from the cells of the blood, I differ widely from all previous observers, with the exception of Seeliger. Concerning the origin of the dorsal tube in the buds of *Perophora*, my results are totally opposed to the conclusion of Pizon, which, as stated above, is not based on sufficient evidence; namely, that "le tube dorsal des Pérophores a la même évolution que le tube dorsal des Botryllidés" (*L.c.*, p. 130). A study of the younger stages would have convinced him of his error. *Perophora viridis*, at all events, presents an exception to the general rule laid down by Pizon that "Chez toutes ces

familles d'Ascidies composées (Clavelinidae, Perophoridae, Botryllidae, Polyclinidae, Distomidae, Didemnidae, et Diplosomidae) l'organe vibratile débute par un tube aveugle, formé par un diverticule de la vésicule endodermique primitive" (*l.c.*, p. 131).

The Sexual Organs.

My observations on the development of the sexual organs have not been carried beyond quite an early stage, but, so far as they go, they closely agree with the description given by Van Beneden and Julin (33) for the buds of *Perophora listeri*.

Shortly after the peribranchial cavity has been completely divided off from the branchial sac, a small collection of cells appears between the two arms of the U-shaped digestive tract, and at the level of the duct of the "organe réfringent," almost at the point where the latter is connected with the stomach. The sexual organs arise from this little spherical mass of cells, which are at first but loosely held together and identical in appearance with the amoeboid cells of the blood. Pl. XXXII, Fig. 32, *a*, which is precisely similar to Van Beneden and Julin's Pl. XVI, Fig. 5, *b*, shows the intimate relation between the cells of the blood and those of the rudiment (*g.r.*), so that there can be no doubt that the two are identical. The connection, already described by the Belgian authors, of some of the peripheral cells of the mass with surrounding blood cells by protoplasmic processes is distinctly seen in the figure at *m.c.* A small, irregular cavity (*c.g.r.*) is also shown in the center of the clump of cells. Some of the free cells lying above the rudiment (Fig. 32, *a*, *g.c.*) are seen to be spindle shaped; these soon become joined, end to end, to form a solid cord, united at one end to the spherical mass of cells and taking a course parallel to that of the intestine (Fig. 32, *b*, *g.c.*). This figure represents a later stage, in which the cavity is considerably enlarged and the genital cord (*g.c.*) is present as a solid single row of cells (*cf.* Pl. XII, Fig. 2, of Van Beneden and Julin).

A furrow, which appears opposite to the attachment of the cord, now divides the hollow sphere into two lobes, the cavities of which are not completely separated, but remain in communi-

cation for a time. In Fig. 32, *c*, the division of the originally simple sphere is seen, but the section is not in the proper plane to show the connection between the two cavities.

One of these lobes gives rise to the testis, the other to the ovary, according to Van Beneden and Julin, who have described in detail how, from the primitive, simple sphere and the single cord of cells, testis, ovary, vas deferens, and oviduct are all differentiated.

My observations would, therefore, seem to support the view of the Belgian authors that the male and female sexual organs do not arise from separate vesicles, as described by Kowalewsky (12) in the buds of *Perophora*, but are formed from one and the same rudiment.

Summary of Results.

(1) The rudiment of the *Perophora* bud, like that of all other ascidians, consists of two vesicles, an outer and an inner one. The former is derived from the ectoderm of the stolon, the latter from the thickened evaginated wall of the stolon partition.

(2) At an early stage the right side of the inner vesicle is found to be much thicker than the remaining portion, and, by a peculiar process of rotatory growth or displacement of the vesicle, is carried down to the ventral side of the bud, where it forms the floor of the pharynx. This process seems to be due to the growth and flattening out of the cells composing the whole wall of the vesicle, except in the thickened region. I am at a loss to explain why the displacement of the vesicle should occur, and to discover the phylogenetic significance of it, if it have any.

(3) The peribranchial sacs arise asymmetrically. As the displacement proceeds, the wall of the inner vesicle is folded in at the point where the right side of the vesicle joins the stolon partition to form the *left peribranchial* sac. The connection of the latter with the partition of the stolon is retained until a much later stage. A longitudinal furrow appearing high up on the right side of the inner vesicle separates off the right

peribranchial sac, and is gradually borne ventrally, as the shifting of the vesicle continues. The constriction of the whole peribranchial cavity eventually takes place in such a way as to cut off from the peribranchial sac a saddle-shaped bag, composed of the median dorsal connecting piece or cloaca and the two lateral portions of the cavity. Anterior and posterior extensions of the latter grow out and surround respectively the anterior and posterior ends of the pharynx. The posterior prolongations are possibly to be regarded as homologous with the epicardial sacs of some other ascidians.

(4) The endostyle appears early as a longitudinal groove in the middle of the thickened portion of the vesicle; from its primitive position on the right side it is moved down to the ventral mid-line by the displacement of the vesicle.

(5) The digestive tract grows out laterally as a blind tube from the posterior end of the inner vesicle high up on the left side. During the change in position of the vesicle its opening into the latter is carried up into the median plane.

The "organe réfringent" arises as a tubular diverticulum from the anterior face of the stomach, and produces a dichotomously branched system of tubules, which surround the intestine and terminate in little dilated vesicles or ampullae.

(6) All the evidence goes to show that the pericardium is formed from free amoeboid cells of the blood. It first appears as a clump of cells adhering to the outer surface of the inner vesicle far up on the right side, and through the shifting of the vesicle is brought down to the ventral side.

(7) The dorsal tube and ganglion are formed only in part from a common rudiment, but there is every reason to believe that both are derived solely from cells of the blood. The former appears as an elongated, solid mass of cells, which lies close against the outer surface of the inner vesicle, a little to the left of the median dorsal line. When the displacement of the vesicle is completed, the rudiment lies in the median plane; it then acquires a lumen, which is put into communication anteriorly with the cavity of the pharynx. The ganglion has a double origin, and is formed by proliferation of the upper wall of the dorsal tube and also by addition of cells of the blood.

(8) The sexual organs have a common origin from free amoeboid blood cells, both testis and ovary arising from one and the same spherical rudiment.

Concluding Remarks.

The results which I have obtained from the study of the budding of *Perophora viridis* furnish additional evidence in support of the view that the development of the bud and that of the embryo do not proceed along parallel lines. The attempts which have been made to harmonize the facts of budding with the germ layer theory have been totally futile in the case of ascidians, and any hypothesis which explains budding in the ascidians as a process of regeneration, by which the organs of the parent, or their germ layers, give rise to similar organs in the bud, must, in the light of known facts, be ruled out.

The rudiment of the bud in all groups of compound ascidians is composed of two vesicles, one within the other, enclosing between them free cells of the blood. The outer vesicle is always derived from the ectoderm of the parent, and gives rise to the ectodermal covering of the bud. The origin of the inner vesicle, however, is not the same in all ascidians. In the Botryllidae it arises, in both embryozooid and blastozooid, from the peribranchial wall, which is formed in the first place from the ectoderm of the embryo. This vesicle is, therefore, ultimately of ectodermal origin in Botryllus, whereas in all other ascidians it comes from an endodermal structure of the parent. Although derived in the two cases from different germ-layers, the inner vesicle may go to form the same organs in the bud but organs which are of widely different origin in the larva; for example, the digestive tract and nervous system (Hjort, Botryllus (8), Distaplia (10), Glossophorum (9)). It is, therefore, perfectly evident that the fate of the inner vesicle entirely precludes the application of the "germ-layer theory" to the ascidian bud.

In *Perophora viridis* the important part played in the bud development by cells of the blood only increases the disagreement. Organs which in other ascidian buds arise from either ectoderm or endoderm are here formed by the coming together

of free amoeboid cells in the body cavity. Such is the origin, for instance, of the nervous system and pericardium, which, however, in the embryo are respectively ectodermal and endodermal structures.

Since in the development of the bud and that of the larva the same end is reached by entirely different roads, and in the former organs do not proceed from corresponding larval organs or even their germ-layers, and, moreover, since in the bud a rudiment derived from one and the same embryonic germ-layer may give rise to structures of widely different nature, one is compelled to believe with Hjort that "die Knospung der zusammen gesetzten Ascidien ein Entwicklungsprocess ist, in welchem sämtliche Organe durch 'Neubildung' aus einer sehr primitiven Anlage entstehen." . . . "Die Knospe muss ihre eigenen Gesetze haben und muss, da sie aus einer wesentlich anderen Anlage hervorgeht als die Larve, auf andere Weise gebildet werden. Ebenso wie das Ei, muss das Material, die Anlage, welche den Ausgangspunkt für die Entwicklung bildet, als ganz undifferenziert gedacht werden und muss alles enthalten, was zur Bildung eines ganzen Individuums nötig ist, ebenso wie die Blastula des Eies" (9; p. 225).

The behavior of the blood cells in the bud development of *Perophora viridis* is of much interest, owing to the number and variety of organs in whose formation they are concerned. As we have seen, these cells give rise to the pericardium, the dorsal tube, the ganglion, and the sexual organs; they also produce the musculature, and, as Kowalewsky (14) and Seeliger (32) have shown in other ascidians, in *Perophora* also they doubtless become the cells of the cellulose test.

As I have repeatedly emphasized in describing the formation of the various organs in question, there is not the slightest discoverable evidence that cells are given off from the wall of the vesicle at the places where such organs arise, or at any other point, for that matter, and the similarity between the cells which make up the different rudiments and the free amoeboid cells of the blood is so perfectly apparent, especially at early stages, that the two are certainly identical.

The origin of these blood cells must, however, be left in

doubt, as it would, of course, be impossible to prove that all or any of them are direct descendants of the embryonic mesoderm, and the most diligent search fails to show that the inner vesicle of the bud is the source from which they are derived. Even though it cannot be shown to be the case, however, it is quite possible that at certain times the primitive vesicle does give off cells which are set free in the blood, and, as this is an undifferentiated structure and plays such an important rôle in the development, the supposition is probable. But, whatever be their origin — whether it is the mesoderm of the embryo or the inner vesicle of the bud-rudiment or both — amoeboid cells floating freely about in the blood spaces become aggregated at certain places and form pericardium, dorsal tube, ganglion, and sexual organs.

A discussion of the nature of these cells and the part played by them in the developing bud will, however, not be out of place. In the case of a fixed organ, like the ganglion or pericardium, which has a definite and determined position, the cells destined to form it must, by virtue of their motile power, come together at the right time and place. Weismann (35; pp. 161, 162), in discussing the process of gemmation in *Clavelina* as described by Seeliger (29), supposes that "these cells contain very different kinds of idioplasm; one, for instance, might contain 'muscle determinants,' and another, 'nerve determinants,' and a third, 'blood corpuscle determinants.'" He further adds that "until we know more of the actual facts concerned, we can only — however unsatisfactory such an assumption may be — attribute to the cells a tendency to become attached at definite points according to the manner in which they have previously been determined."

It seems to me, however, that the opposite assumption, which Weismann regards as less likely, namely, "that these cells develop into muscle, nerve, or sexual cells, according to their point of attachment," is more in accordance with the facts presented by the bud development of *Perophora viridis*.

We have already seen in the very young bud, when it consists merely of two simple layers, and before there is the slightest indication of the appearance of any organs, that cells

are attached in many places to the inner surface of the ectoderm and outer surface of the endoderm, but that they are not more numerous at any one spot than another (Pl. XXX, Figs. 7, 8). Their power of amoeboid movement over any surface with which they come in contact would account for their presence on the walls of the vesicles.

It would seem more probable that these cells are all alike and indifferent, and that the nature of the organs to which they give rise is determined, not by any prearranged condition of their idioplasm, but by the particular point to which they happen to become attached. I regard it as a significant fact that cells are found, not only at the places where organs will arise, but also at many other points. Those of the cells which chance to fall, as it were, on fertile soil will undergo further development, and, under the formative influence exerted upon them by that portion of the wall to which they adhere, will be utilized in building up a definite structure.

All parts of the walls cannot possess a specific determining power, and such cells as lodge on barren ground are not further modified, and do not furnish material for the formation of organs.

According to this view, one of these cells is the equivalent of any other, and it is only a few that find favorable positions and have their latent possibilities called forth. Those which become attached at a point high up on the right side of the inner vesicle in the posterior region of the bud will form the pericardium; others on the dorsal side, at the anterior end, will give rise to the dorsal tube, and still others, which lodge on the upper wall of the latter, will help to construct the ganglion; some adhere to the inner surface of the ectoderm, lengthen out, and become muscle fibers; some wander through the ectoderm, and on the external surface are transformed into the cells of the cellulose test; while others find a definite place in the posterior region of the bud and develop into the sexual organs.

This view is opposed to the supposition of Seeliger spoken of above, that in *Clavelina* the ganglion of the bud is formed from free cells of the blood which had earlier composed the larval ganglion and been liberated on the dissolution of that

organ; these cells would, therefore, have already possessed a ganglionic nature, and would merely resume in the bud their former function.

On any such assumption, it is almost impossible to imagine how isolated specific cells, moving freely about in the blood, could reach their proper destination. On the reverse assumption, however, the presence of these cells at any particular point is accidental, but, once there, their potentialities are called out under the specific formative influence of the place of attachment.

BALTIMORE, MD.,
April 18, 1896.

APPENDIX.

Since the foregoing was written an article by Prof. W. E. Ritter has appeared in this journal (Vol. XII, No. 1) on the budding of *Goodsiria* and *Perophora*. Of the interesting account given of the budding in the former genus I have nothing to say, but, as the results which he has obtained from his study of the process in *Perophora* differ in some particulars from my own on the same genus, I desire to add another word.

After reading his paper I again examined my sections with exceptional care under an oil-immersion lens, but am more firmly convinced than ever that the origin of the pericardium and dorsal tube and ganglion, points on which Ritter and I disagree, is that which I have described above; and that these structures in *Perophora viridis*, at all events, do arise *solely* from free amoeboid cells of the blood. I am confident that in the form I have studied no cells are given off *directly* from the wall of the inner vesicle to the rudiments of the organs in question, as no cell proliferation can be observed, and never is there the slightest interruption in the boundary line of the wall.

Although the peculiar rotation or transverse shifting of the inner vesicle, which I have described as occurring in *Perophora viridis*, does not take place to as great an extent in *P. annectens*, it nevertheless is found, and in this respect Ritter has fully confirmed my observations. Some time before the appearance

of his completed paper he published a preliminary note,¹ in which he stated that "when the differentiation of the 'endoderm' into the branchial and two peribranchial sacs takes place, it does so in such a way that the developing blastozoöid is connected with the double-walled partition of the stolon, not by the *branchial sac*, as has been hitherto supposed, but by the left peribranchial sac"; but no mention was made of the rotation of the vesicle by means of which this condition is brought about.

Shortly afterwards, a brief account² of my results on *P. viridis* was published, in which I described the rotation of the inner vesicle and showed by means of figures that this process causes the septum of the stolon to become connected with the left peribranchial and not the branchial sac. I have repeated this statement, because from the account given by Ritter of the relation of the bud to the stolon (*loc. cit.*, pp. 190, 192) one would infer that all my observations were merely a confirmation of his, as he says, "Lefevre fully confirms my results in this particular"; whereas, before the appearance of my note, the above quotation, which affords no explanation of the process which brings about the connection of the septum with the left peribranchial sac, was the only published statement of his on the subject.

I am fully aware of the danger of inferring that, because something takes place in a certain way in the bud development of one ascidian, the same is true of another, however closely the two forms may be related, and in the light of all we know concerning the budding of different ascidians it is not surprising to find even great differences. It is, therefore, perfectly possible that both Ritter and myself are correct in our observations on the derivation of the pericardium and dorsal tube in the two species of *Perophora* which we have studied; and that in the one these organs are formed both by cells given off *directly* from the wall of the vesicle to their rudiments, and also by free cells of the blood, while in the other it is from this latter source alone that they arise. Ritter's account, however, is not abso-

¹ *Anat. Anz.*, Bd. x, No. 11.

² *Johns Hopkins University Circulars*, No. 119, June, 1895.

lately convincing to my mind, and, in fact, he himself states (*loc. cit.*, p. 197) that his "conclusion rests upon a *preponderance of evidence*," as there is certainly evidence which is "indicative of a mesenchymal origin of the cells." I do not consider as valid his argument that the connection between the wall of the inner vesicle and the pericardial rudiment is too strong to be a mere contact, and cannot be supposed to be a secondary one, as a complete separation takes place later. A glance at Pl. XXXII, Figs. 25, *a*, *b*, and 26, *a*, *b*, of this paper will show that, even granting for the moment that the cells of these two rudiments have been given off from the vesicle, they are very loosely attached at this early stage to the wall, and that, however intimate the connection between the two may become later, it must necessarily be a secondary one. In *P. viridis* I believe it to be merely a contact, although a firm one, and as the pericardial sac ultimately breaks away from the vesicle, on *a priori* grounds alone one would not expect to find an organic union; I have the additional evidence that I have been unable to discover any interruption in the boundary line of the vesicle, there being everywhere a sharp demarcation between the wall and the rudiment. Any argument from this source must, therefore, be ruled out, for it is certainly true that a firm secondary contact is not only possible, but actually does occur in *P. viridis*.

It is to be regretted that Ritter did not observe earlier stages in the development of the pericardium and dorsal tube, but his Figs. 68 and 74, and the statement on page 197 in regard to the pericardium, that he has found "no sections in which *at some places* I cannot see the separating line to be interrupted," strongly incline one to accept his conclusions for *P. annectens*.

I share the belief with Ritter that there is very good ground for holding that the inner vesicle gives off cells into the body space, and is, therefore, one source, at all events, of mesenchyme cells, which are found free in the blood, and apparently take part in the formation of the pericardium and dorsal tube. It is hardly possible to suppose, as he states, that these cells are all derived from the mesenchyme of the embryoöid, and, in fact, the inner vesicle of the buds is about the only place where one could look for their origin. I do not consider, however, that

the Figs. 77, 78, which he gives in support of his statement that he believes he has observed instances where this is taking place, are cases in point; it does not seem to me that there is any evidence that the cells marked *b.c'* have come from the vesicle, as there is no connection between the two, and they might simply be outside cells lying in depressions of the wall.

I have observed no cases in *P. viridis* where I could be positively sure that cells of the blood were derived in this way, but I regard it as extremely probable that at certain times or in certain buds at least such a process does occur, for it is difficult to imagine where these cells come from if not from the inner vesicle. If this be true, then the pericardium and dorsal tube in *P. viridis* are ultimately derived from the vesicle, and in *P. annectens* those cells which are directly given off into the rudiments of these structures and the blood cells which aid in their formation are cells of the same kind, having come in both cases from the wall of the vesicle of the same bud or a sister bud.

I have recently had the opportunity of studying the bud development in another genus of the Clavelinidae, namely, *Ecteinascidia*, Herdman, and in this form I have observed undoubted instances where cells are passing out from the vesicle to be set free in the blood; they are simply budded off into the body space, and the appearance is quite different from that of Ritter's Figs. 77, 78. A brief account of the development will shortly appear in the *Anatomischer Anzeiger*, but I may state here that I have found the dorsal tube, pericardium, and sexual organs to be all formed in large part by cells which are given off *directly* from the wall of the inner vesicle to their rudiments, but which are unquestionably supplemented by amoeboid cells from without. Here, therefore, the two processes do occur without a doubt, but, as all the cells which are concerned in the formation of these organs are derived from the same source—some, however, only indirectly—there is no essential difference between them.

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DESCRIPTION OF THE PLATES.

All the drawings have been made with a camera lucida. The lenses used were those of Zeiss, and the magnification is given after the description of each figure.

REFERENCE LETTERS.

<i>a.</i>	anus.	<i>int.</i>	intestine.
<i>amp.</i>	ampulla.	<i>in.v.</i>	inner or primitive vesicle.
<i>bl.s.</i>	blood sinus of stolon.	<i>l.a.ex.</i>	anterior extension or pouch of left peribranchial sac.
<i>br.o.</i>	branchial orifice.	<i>l.d.t.</i>	lumen of dorsal tube.
<i>br.s.</i>	branchial sac.	<i>l.p.ex.</i>	posterior extension or pouch of left peribranchial sac (left epicardial sac?).
<i>br.w.</i>	branchial wall.	<i>l.pb.s.</i>	left peribranchial sac.
<i>c.g.r.</i>	cavity of rudiment of sexual organs.	<i>m.c.</i>	free amoeboid cells of blood.
<i>cl.</i>	cloaca or atrium.	<i>ml.c.</i>	muscle-cells.
<i>cl.o.</i>	cloacal orifice.	<i>o.e.</i>	oesophagus.
<i>c.p.c.r.</i>	cavity of pericardial rudiment.	<i>o.r.</i>	"organe réfringent" or pyloric gland.
<i>c.pt.</i>	cavity of stolon double-walled partition.	<i>o.r.d.</i>	duct of "organe réfringent."
<i>c.t.</i>	cells of cellulose test.	<i>o.r.r.</i>	rudiment of "organe réfringent."
<i>d.t.</i>	dorsal tube or hypophysis.	<i>pb.w.</i>	peribranchial wall.
<i>d.t.r.</i>	rudiment of dorsal tube.	<i>pc.</i>	pericardium.
<i>d.w.pb.s.</i>	thickened dorsal wall of peribranchial sac, which invaginates to form the heart.	<i>pc.r.</i>	pericardial rudiment.
<i>e.br.o.</i>	ectodermal invagination to form the branchial orifice.	<i>pt.</i>	double-walled partition or cloison of stolon.
<i>ec.</i>	ectoderm.	<i>r.a.ex.</i>	anterior extension or pouch of right peribranchial sac.
<i>e.cl.o.</i>	ectodermal invagination to form the cloacal orifice.	<i>r.p.ex.</i>	posterior extension or pouch of right peribranchial sac (right epicardial sac?).
<i>end.</i>	endostyle.	<i>r.pb.s.</i>	right peribranchial sac.
<i>f.l.pb.s.</i>	fold which forms left peribranchial sac.	<i>r.st.c.</i>	remnant of connection between the stolon partition and left peribranchial sac.
<i>f.r.pb.s.</i>	fold which forms right peribranchial sac.	<i>r.w.in.v.</i>	thickened wall of inner vesicle on right side.
<i>g.c.</i>	genital cord.	<i>st.</i>	stomach.
<i>gl.</i>	ganglion.	<i>st.c.</i>	connection of stolon partition with inner vesicle or with left peribranchial sac.
<i>gl.r.</i>	rudiment of ganglion.	<i>st.w.</i>	wall of stomach on anterior side.
<i>g.r.</i>	rudiment of sexual organs.	<i>stl.</i>	stolon.
<i>g.s.</i>	branchial stigmata or gill slits.	<i>t.</i>	cellulose test.
<i>g.s.r.</i>	rudiment of branchial stigmata.		
<i>gt.</i>	digestive tract.		
<i>gt.r.</i>	rudiment of digestive tract.		
<i>ht.r.</i>	rudiment of heart.		

EXPLANATION OF PLATE XXIX.

All the figures of this plate were drawn from specimens mounted as whole objects. The test is only represented in Fig. 1. The buds were stained slightly in borax carmine, and were perfectly transparent.

FIG. 1. Shows evagination of ectoderm and partition of stolon to form the bud-rudiment. $\times 170$.

FIG. 2. Slightly older rudiment, seen from right side. $\times 170$.

FIG. 3. Still older rudiment, seen from right side, and showing anterior elongation and constriction of the stolon connection. Rudiment of dorsal tube is shown; that of pericardium is present, but is not drawn in the figure. $\times 170$.

FIG. 4. Young bud, seen from right side, at a stage when development of peribranchial cavity, pericardium, and digestive tract is well advanced. $\times 170$.

FIG. 5. Bud considerably older than the last, showing extensions of peribranchial sacs, formation of branchial stigmata, and further development of digestive tract, pericardium, etc. $\times 170$.

EXPLANATION OF PLATE XXX.

The test is only shown in Figs. 6 and 7, and in Figs. 9-15 the ectoderm is represented merely by a line. The elements of the blood have not been drawn in Figs. 10-15.

FIG. 6. Transverse section of stolon, showing double-walled partition, *pt.* $\times 500$.

FIG. 7. Transverse section of stolon at point where a bud is beginning to form. The ectoderm on one side is thickened, and the double-walled partition is much thickened and dilated. $\times 500$.

FIG. 8. Transverse section through a very young bud-rudiment and stolon, at about the stage of Fig. 1, Pl. XXIX. The walls of the partition are coming together to close off the inner vesicle, *in.v.* Blood cells are found lying against the outer surface of the latter. $\times 500$.

FIG. 9. Transverse section through posterior end of a bud-rudiment at about the stage of Fig. 3, Pl. XXIX, showing thick wall of inner vesicle on right side, *r.w.in.v.*, and beginning of pericardial rudiment, *pc.r.* $\times 500$.

FIG. 10. Transverse section through posterior end of a young bud, showing an early stage in the displacement or shifting of the inner vesicle. The wall of the latter is being bent in at the point indicated by the line *a*. $\times 200$.

FIG. 11. Transverse section through posterior end of bud slightly older than last, showing further progress of the displacement of the vesicle. The pericardial rudiment, *pc.r.*, is at a much lower level than in the last figure, and the wall of inner vesicle is being bent in further at *a*; this is the fold which will form the left peribranchial sac, *fl.pb.s.* $\times 200$.

FIG. 12. Transverse section through the middle region of same bud as the one shown in Fig. 11. The section lies in front of the connection with the stolon partition, and shows the beginning of the fold which will form the right peribranchial sac, *f.r.pb.s.* The rudiment of dorsal tube, *d.t.r.*, is also shown. $\times 200$.

FIG. 13. Transverse section through the anterior end of a bud slightly older than Fig. 9. The rudiment of the dorsal tube is shown, and consists at this stage of a few scattered cells, adhering to the wall of the inner vesicle a little to the left of the median dorsal line, *d.t.r.* The difference in thickness between the right side and the rest of the vesicle is seen to be but slight in this region. $\times 200$.

FIG. 14. Median sagittal section of a bud at about the stage of Fig. 4, Pl. XXIX, showing the position and extent of the dorsal tube, *d.t.* The peribranchial cavity is not yet completely constricted off from the branchial sac. $\times 200$.

FIG. 15. Transverse section through the extreme posterior end of a bud, showing the origin of the gut, *gt.r.*, as a diverticulum on the left side of the branchial sac. $\times 200$.

EXPLANATION OF PLATE XXXI.

In all the figures of this plate the ectoderm is represented by a line, and the elements of the blood have been omitted for the sake of simplicity.

FIGS. 16-21. Series of transverse sections of a bud at about the same stage as that shown in Fig. 4, Pl. XXIX. The sections are taken respectively at the levels indicated by the lines *a*, *b*, *c*, *d*, *e*, and *f*, in Fig. 4. Fig. 16, line *a*, the most anterior section, shows undivided portion of branchial sac, *br.s.*, and dorsal tube, *d.t.* Fig. 17, line *b*, is a little further back, and shows anterior extension of right peribranchial sac, *r.a.ex.* Fig. 18, line *c*, is taken immediately in front of the cloacal cavity, and shows the extensions of the peribranchial cavity on both sides. The section passes through the extreme posterior end of the dorsal tube, *d.t.* Fig. 19, line *d*, passes through the anterior end of the cloacal cavity, *cl.*, which is seen to connect the lateral portions of the peribranchial cavity. Fig. 20, line *e*, is taken from the posterior end of the bud, and shows the connection of the left peribranchial sac with the stolon partition at *st.c.* The pericardial sac, *pc.*, is also shown in this region. Fig. 21, line *f*, shows the undivided extreme posterior end of the branchial sac. The section passes through a portion of the intestine, *int.*, and behind the connection with the stolon partition. $\times 200$.

FIGS. 22-24. Series of transverse sections of a bud of about the same age as the one represented in Fig. 5, Pl. XXIX. Fig. 22 is a section through the anterior region, and shows the extensions of the peribranchial sacs, *r.a.ex.* and *l.a.ex.* Fig. 23 is drawn from a section which is taken from the middle region of the bud, and shows the median portion or cloaca, *cl.*, connecting the lateral divisions of the peribranchial cavity, *r.pb.s.* and *l.pb.s.*, which is now entirely cut off from the branchial sac, *br.s.* Fig. 24 shows the posterior extensions (epicardial sacs?) of the peribranchial cavity, *r.p.ex.* and *l.p.ex.*, and the remnant of the connection with the stolon partition, *r.st.c.* $\times 200$.

EXPLANATION OF PLATE XXXII.

FIG. 25, *a, b, c, d,* and *e*. Sections illustrating the development of the pericardium. *a* is from a frontal section at a very young stage, and shows the rudiment when it consists merely of a few cells loosely attached to the inner vesicle. The remaining figures are drawn from transverse sections. In *b* and *c* the rudiment has increased in size; cells are being added from without, and nuclei within the mass are seen to be dividing. In *d* and *e* a cavity, *c.p.c.r.*, has appeared in the center of the rudiment, which is now much larger, and the cells are becoming arranged into a one-layered epithelium. $\times 700$.

FIG. 26, *a, b, c, d,* and *e*. Transverse sections representing stages in the development of the dorsal tube. In *a* a few cells are seen loosely grouped together, and lying on the wall of the inner vesicle. In *b* the association with surrounding blood cells is apparent. In *c* and *d* the mass has become compact, and cell-boundaries have entirely disappeared; in the latter figure the lumen in the center has begun to form, *l.d.t.* In *e* the tube is completely formed, and the cells are arranged around the lumen in a one-layered epithelium. $\times 700$.

FIG. 27, *a, b, c, d,* and *e*. Transverse section showing the development of the ganglion. In *a* and *b* a few cells resembling amoeboid cells of the blood are seen lying on the dorsal side of the dorsal tube, *d.t.* The outer membrane of the latter is broken at this point, and nuclei appear to be wandering out into the rudiment, *gl.r.* In *c* and *d* the rudiment is much enlarged, and in the latter the nuclei are beginning to arrange themselves peripherally. In *e* the wall of the dorsal tube under the ganglion has been re-formed, and the central portion of the latter is now free from nuclei and occupied by fine fibrils. $\times 700$.

FIG. 28, *a* and *b*. Stages in the formation of the branchial orifice. In *a* the much thickened invaginated ectoderm, *e.br.o.*, is seen nearly touching the branchial wall. The fusion of the two walls is shown in *b*. $\times 700$.

FIG. 29, *a* and *b*. These sections illustrate the development of the branchial stigmata. In *a* the thickened branchial wall, *br.w.*, is slightly evaginated, and is in contact with the thickened patch of cells in the visceral wall of the peribranchial sac, *pb.w.* The lower portion of *b* shows the fusion between the two walls, and the upper portion, a stage after the formation of the opening. $\times 700$.

FIG. 30. Section through the wall of the stomach, *st.w.*, and a portion of the duct of the "organe réfringent," *o.r.r.*, showing clearly the connection of the latter with the digestive tract. $\times 700$.

FIG. 31. Transverse section of the terminal portion of the intestine, *int.*, showing the surrounding ducts and ampullae of the "organe réfringent," with their deeply stained nuclei, *amp.* $\times 700$.

FIG. 32, *a, b,* and *c*. Sections illustrating the early development of the sexual organs. In *a* an early stage is shown when the rudiment, *g.r.*, is merely a loose mass of cells surrounding a slight cavity, *c.g.r.* A couple of the elongated cells which will help to form the genital cord are seen at *g.c.* In *b* the cavity of the rudiment is much enlarged, and the genital cord is prominent, and attached to the sphere; in both these figures the genital rudiment is seen to lie close to the wall of the digestive tract. In *c* an older stage is shown, in which the cavity of the sphere has become divided into two, *c* and *c'*; the genital cord, although only represented in part, now extends nearly to the posterior wall of the cloaca, and lies close to and parallel with the intestine. $\times 700$.